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Abstract: A low high-density lipoprotein (HDL) plasma concentration and the abundance of small dense low-density lipoproteins (LDL) are risk factors for developing type 2 diabetes. We therefore investigated whether HDL and LDL play a role in the regulation of pancreatic islet cell apoptosis, proliferation and secretory function. Isolated mouse and human islets were exposed to plasma lipoproteins of healthy human donors. In murine and human beta-cells LDL decreased both proliferation and maximal glucose-stimulated insulin secretion. The comparative analysis of beta-cells from wild type and LDL receptor deficient mice revealed that the inhibitory effect of LDL on insulin secretion but not on proliferation requires the LDL receptor. HDL was found to modulate the survival of both human and murine islets by decreasing basal as well as IL-1beta and glucose induced apoptosis. IL-1beta induced beta-cell apoptosis was also inhibited in the presence of either the delipidated protein or the deproteinated lipid moieties of HDL, apolipoprotein A1 (the main protein component of HDL) or sphingosine-1-phosphate (a bioactive sphingolipid mostly carried by HDL). In murine beta-cells, the protective effect of HDL against IL-1beta induced apoptosis was also observed in the absence of the HDL receptor scavenger receptor class B type 1 (SRB1). Our data show that both LDL and HDL affect function or survival of beta-cells and raise the question whether dyslipidemia contributes to beta-cell failure and hence the manifestation and progression of type 2 diabetes mellitus.

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Low- and High-Density Lipoproteins Modulate Function, Apoptosis, and Proliferation of Primary Human and Murine Pancreatic β-Cells

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A low high-density lipoprotein (HDL) plasma concentration and the abundance of small dense low-density lipoproteins (LDL) are risk factors for developing type 2 diabetes. We therefore investigated whether HDL and LDL play a role in the regulation of pancreatic islet cell apoptosis, proliferation, and secretory function. Isolated mouse and human islets were exposed to plasma lipoproteins of healthy human donors. In murine and human β-cells, LDL decreased both proliferation and maximal glucose-stimulated insulin secretion. The comparative analysis of β-cells from wild-type and LDL receptor-deficient mice revealed that the inhibitory effect of LDL on insulin secretion but not proliferation requires the LDL receptor. HDL was found to modulate the survival of both human and murine islets by decreasing basal as well as IL-1β and glucose-induced apoptosis. IL-1β-induced β-cell apoptosis was also inhibited in the presence of either the delipidated protein or the deproteinated lipid moieties of HDL, apolipoprotein A1 (the main protein component of HDL), or sphingosine-1-phosphate (a bioactive sphingolipid mostly carried by HDL). In murine β-cells, the protective effect of HDL against IL-1β-induced apoptosis was also observed in the absence of the HDL receptor scavenger receptor class B type 1. Our data show that both LDL and HDL affect function or survival of β-cells and raise the question whether dyslipidemia contributes to β-cell failure and hence the manifestation and progression of type 2 diabetes mellitus. **(*Endocrinology* 150: 4521–4530, 2009)**

The development of type 2 diabetes is characterized by a decrease in the functional β-cell mass that can no longer compensate for insulin resistance (1, 2, 3). Patients with type 2 diabetes display, along with hyperglycemia, elevated circulating levels of free fatty acids, triglycerides, and small dense low-density lipoproteins (LDL) particles as well as low plasma levels of high-density lipoprotein (HDL) cholesterol and its major protein component apolipoprotein (Apo)-A1 (4). Even more so, low HDL cholesterol, hypertriglyceridemia, and small dense LDL often precede the manifestation of type 2 diabetes and epidemiological studies have shown that low HDL cholesterol is an independent risk factor for developing type 2 diabetes (5). Recent data in rodent β-cells have indicated a role for lipoproteins in the regulation of islet cell survival and insulin synthesis suggesting a causal or

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Abbreviations: Apo, Apolipoprotein; Bcl2, B-cell leukemia-2; BrdU, 5-bromo-2′-deoxyuridine; C, cholesterol; DAPI, 4′,6-diamidino-2-phenylindole; FLIP, Fas-associated death domain-like IL-1β converting enzyme inhibitory protein; HDL, high-density lipoprotein; iNOS, inducible nitric oxide synthase; LDL, low-density lipoprotein; LDLR, LDL receptor; pHDL, protein moiety of HDL; SOCS, suppressor of cytokine signaling; S1P, sphingosine-1-phosphate; SRB1, scavenger receptor B1; TUNEL, terminal deoxynucleotidyl-transferase-mediated deoxyuridine 5-triphosphate nick-end labeling.

modulatory role of lipoproteins in the precipitation of type 2 diabetes (6, 7).

In fact, primary murine β-cells as well β-cell lines such as βTC3 were found to express various lipoprotein receptors including members of the LDL receptor gene family and the scavenger receptor B gene family. In addition, both LDL and HDL were found to modulate the survival of these cells. High concentrations of native LDL as well as oxidatively modified LDL decreased the proliferation and increased apoptosis of β-cells (6–8). In addition, mice with a conditional knockout of the ATP binding cassette transporter A1 in pancreatic β-cells were found to be glucose intolerant due to cholesterol overloading of β-cells and insufficient insulin secretion (9).

This study was undertaken to examine the effects of LDL and HDL on the survival, proliferation, and function of primary human and mouse islet cells and unravel the lipoprotein components and lipoprotein receptors that mediate any of these effects. We found that prolonged exposure to LDL impairs insulin secretion in a possibly LDL receptor-dependent manner and decreases human and mouse islet cell proliferation independently of the LDL receptor. To unravel any protective effects of HDL we challenged islet cells with glucose or IL-1β as known diabetogenic factors (10, 11). Under this condition entire HDL particles as well as isolated ApoA1 and sphingosine-1-phosphate (S1P), a bioactive lipid mostly carried by HDL (12), were found to mediate these protective effects. The antiapoptotic effect of HDL did not require expression of the scavenger receptor B1 (SRB1).

Materials and Methods

Isolation of HDL, LDL, and HDL protein and lipid moieties

Blood was collected from healthy donors. Plasma LDL and HDL fractions were isolated by sequential ultracentrifugation (LDL: 1.006 < LDL density < 1.063; HDL: 1.063 < HDL density < 1.21), dialyzed against LDL buffer [NaCl 1.5 M, EDTA 3 mM (pH 7.4)], and finally filter sterilized using a 0.22 μm microfilter (13). Cholesterol concentrations of the isolated lipoproteins were measured using the Cholesterol Oxidase Phenol 4-Aminoantipyrine Peroxidase method on a Cobas modular autoanalyzer (Roche Diagnostics, Rotkreuz, Switzerland) and used for the calibration of lipoprotein amounts given to the cells. On average, 1 mM LDL cholesterol (C) and HDL-C correspond to 0.36 and 1.5 mg/ml protein, respectively. The purity of the lipoprotein preparation was verified by apolipoprotein composition analysis in a 10% SDS-PAGE to exclude contaminations with LDL or albumin in the HDL fraction and to exclude contamination with HDL or albumin in the LDL fraction. HDL and LDL from different donors have never been pooled and were always used rapidly after isolation to prevent any modification or degradation. The lipid-free protein and protein-free lipid moieties of HDL were extracted and purified as described previously (14, 15). The protein-free lipid moiety was bound to 1% fatty acid-free BSA (Sigma-Aldrich, St. Louis, MO) before incubation with the islet cells. One percent BSA did not influence mouse islet cell survival.

Islet isolation and culture

Human islets were isolated from pancreata of organ donors at the University of Geneva Medical Center. Human islets were cultured in CMRL-1066 medium containing 5 mmol/liter glucose, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% fetal calf serum (Invitrogen, Basel, Switzerland). Islets from 20 different donors (11 males, nine females) were used for this study (average age 49 yr; average body mass index 27 kg/m^2). Mouse islets were isolated from C57BL/6J mice, LDL receptor-deficient (LDLR$^{-/-}$) mice (Jackson Laboratory, Bar Harbor, ME) and SRB1-deficient (SRB1$^{-/-}$) mice (16) by collagenase digestion (Worthington Biochemical Corp., Lakewood, NJ) and separated from the exocrine tissue by density gradient (Histopaque-1119; Sigma-Aldrich) as previously described (17). Islets were further handpicked before plating for higher purity. Mouse islets were cultured in RPMI 1640 medium containing 11 mmol/liter glucose, 40 μg/ml gentamicin, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% fetal calf serum (Invitrogen). Islets were cultured on extracellular matrix-coated plates (20 islets/plate) derived from bovine corneal endothelial cells (Novamed, Jerusalem, Israel). The islets cultured on extracellular matrix-coated plates were left in islet media for 48 h to adhere and spread before initiation of the experiments. To obtain single islet cells, freshly isolated mouse islets were trypsinized and mechanically dispersed as previously described (18). The single cells were cultured on extracellular matrix-coated plates. Islets were treated with glucose, IL-1β (R&D, Minneapolis, MN), HDL, LDL, human APOA1 (Calbiochem, San Diego, CA), or sphingosine-1-phosphate (Avanti Polar Lipids, Alabaster, AL) at concentrations reported in the results section. Sphingosine-1-phosphate was added to the cells coupled to 1% fatty acid-free BSA (Sigma-Aldrich).

Insulin secretion

For acute insulin release in response to glucose, islets (20 islets/plate) were washed and incubated in Krebs-Ringer buffer containing 2.8 or 16.7 mmol/liter glucose and 0.5% BSA for 1 h. Islet insulin was extracted with 0.18 mol/liter HCl in 70% ethanol for determination of insulin content. Secreted insulin and insulin content was assayed by RIA (CIS Bio International, Gif-sur-Yvette, France) (17). For each condition in each individual experiment, three plates with 20 islets each were treated. Each n represents a different human islet donor or different mouse islet isolation and a different plasma donor.

Proliferation and apoptosis of islet cells

For human islet cell proliferation studies, a monoclonal antibody against the human Ki-67 antigen (monoclonal mouse anti Ki-67 prediluted, incubation: 1 h at room temperature; Zymed Laboratories, San Francisco, CA) was used (secondary antibody: Histostain Plus kit, Zymed Laboratories). For mouse islet cell proliferation studies, 5-bromo-2′-deoxyuridine (BrdU) was added to the mouse islets at the start of the treatment. The incorporated BrdU was detected with an anti-BrdU antibody (dilution: 1:10, incubation: 30 min at 37 C; Roche, Basel, Switzerland; secondary antibody: donkey antimouse Cy3 (715-165-150), dilution: 1:50, incubation: 30 min at 37 C; Jackson Im-

munoResearch, Baltimore, MD). The free 3′-OH strand breaks resulting from DNA degradation were detected by the terminal deoxynucleotidyl-transferase-mediated deoxyuridine 5-triphosphate nick-end labeling (TUNEL) technique according to the manufacturer's instructions (*in situ* cell death detection kit; Roche). For mouse islet single cell proliferation studies, BrdU was added to the single cells at the start of the treatment. The incorporated BrdU was detected with an anti-BrdU antibody (dilution: 1:10, incubation: 30 min at 37 C; Roche; secondary antibody: donkey antimouse Cy3 (715-165-150), dilution: 1:50, incubation: 30 min at 37 C; Jackson ImmunoResearch) and the single cells were costained with a guinea pig anti-insulin antibody (dilution :1:50, incubation: 30 min at 37 C; Dako, Glostrup, Denmark; secondary antibody: donkey anti-guinea pig fluorescein isothiocyanate (706-096-148), dilution: 1:50, incubation: 30 min at 37 C, Jackson ImmunoResearch). The numbers of apoptotic or proliferative cells were determined by careful counting with a fluorescent microscope (Axioplan 2; Zeiss, Jena, Germany) as previously described (18). For each condition in each individual experiment, 50 islets were analyzed. Each n represents a different human islet donor or different mouse islet isolation and a different plasma donor.

Confocal imaging of β-cell apoptosis

Mouse islets were costained for apoptosis (TUNEL, as described above), insulin (dilution: 1:50, incubation: 30 min at 37 C; Dako; secondary antibody: donkey anti-guinea pig Cy5 (706-175-148), dilution: 1:100, incubation: 30 min at 37 C; Jackson ImmunoResearch), and nuclei [4′,6-diamidino-2-phenylindole (DAPI), dilution 1:1000, incubation: 30 min at 37 C]. Multiple labeling immunofluorescence images of mouse islets were captured using a scanning confocal head coupled to an upright microscope (TCS SP2; Leica, Wetzlar, Germany) with UV light and Ar-Kr laser using 358, 488, and 647 nm excitation and 63×/1.40 oil PlanApo objective lens. For each islet series, eight pictures, corresponding to eight different z planes, were acquired with Leica software and merged. The series allowed the determination of the number of apoptotic β-cells and non-β-cells for each islet. For each condition in each individual experiment, 30–40 islets were analyzed. Each n represents a different human islet donor or different mouse islet isolation and a different plasma donor.

RNA extraction and real-time PCR

Total mouse islet RNA was extracted from 100 islets plated on extracellular matrix-coated culture dishes with a total RNA extraction kit (Nucleospin RNAII; Marchery Nagel, Düren, Germany) and reverse transcribed with random hexamers as previously published (19). Commercially available mouse primers to 18s rRNA, Fas, Fas-associated death domain-like IL-1β converting enzyme inhibitory protein (FLIP), inducible nitric oxide synthase (iNOS), suppressor of cytokine signaling (SOCS)-3, and B-cell leukemia-2 (Bcl2) were purchased and assayed according to the manufacturer's protocol using the ABI 7000 system (Applied Biosystems, Foster City, CA). Changes in mRNA expression were calculated using the difference of cycle threshold values.

Statistical analysis

Data are expressed as means ± SEM, with the number of individual experiments presented in the figure legends. Data were normalized with the control set to 1. All data were tested for normality and analyzed with PRISM (GraphPad, San Diego, CA). Significance was tested using Student's *t* test and ANOVA with Bonferroni *post hoc* test for multiple comparison analysis. Significance was set as $P < 0.05$.

Results

LDL decreases proliferation and function of murine and human β-cells

Human and mouse islets were cultured on extracellular matrix-coated plates for 4 d in the presence of increasing concentrations of LDL-C. Already LDL-C concentrations as low as 0.8 and 1 mM (as defined by cholesterol concentration) decreased the proliferation of mouse and human islet cells by 50 and 70%, respectively. Higher concentrations did not aggravate the antiproliferative effect of LDL in either murine or human islets (Fig. 1, A and B). Furthermore, as determined by counting of single islet cells costained for insulin and BrdU (Fig. 1G), the decrease in islet cell proliferation was found to occur in β-cells. Already after 2 d incubation, 3.1 mM LDL-C decreased mouse islet cells proliferation (Fig. 1H). However, the antiproliferative effect of LDL was milder after 2 d than after 4 d incubation. LDL did not cause apoptosis of mouse or human islet cells (Fig. 1, C and D). To rule out that LDL particle modification (oxidation) during the incubation time was responsible for the observed effect, the experiments were repeated with media and LDL changed and added fresh every day during the 4-d culture period. Under these conditions LDL still decreased basal mouse islet cell proliferation by 50% (control, 5.6 ± 1.8 and 3.1 mM; LDL, 2.5 ± 0.7 BrdU-positive cells/islet, n = 6, $P < 0.05$). Similarly, in the presence of a cocktail of antioxidants (vitamin A, 1 μM; vitamin E, 0.5 μM; dithiothreitol, 10 μM), LDL also decreased basal mouse islet cell proliferation during 4 d of incubation (control, 0.6 ± 0.12 and 3.1 mM; LDL, 0.09 ± 0.04 BrdU-positive cells/islet, n = 3, $P < 0.05$). Furthermore, in human and mouse islets 3.1 and 6.2 mM, LDL-C decreased maximal glucose-stimulated insulin secretion (Fig. 1, E and F). Based on the results described here, we used LDL at physiological concentrations of 3.1 mM, for subsequent experiments.

The LDLR mediates the inhibitory effect of LDL on insulin secretion but not its inhibitory effect on islet cell proliferation

To investigate whether the LDLR mediates the inhibitory effects of LDL on β-cell proliferation and insulin secretion, islets isolated from $LDLR^{-/-}$ and wild-type mice were exposed for 4 days to LDL. LDL decreased basal proliferation, independently of LDL receptor expression (Fig. 2, A and B). By contrast the trend toward an impair-

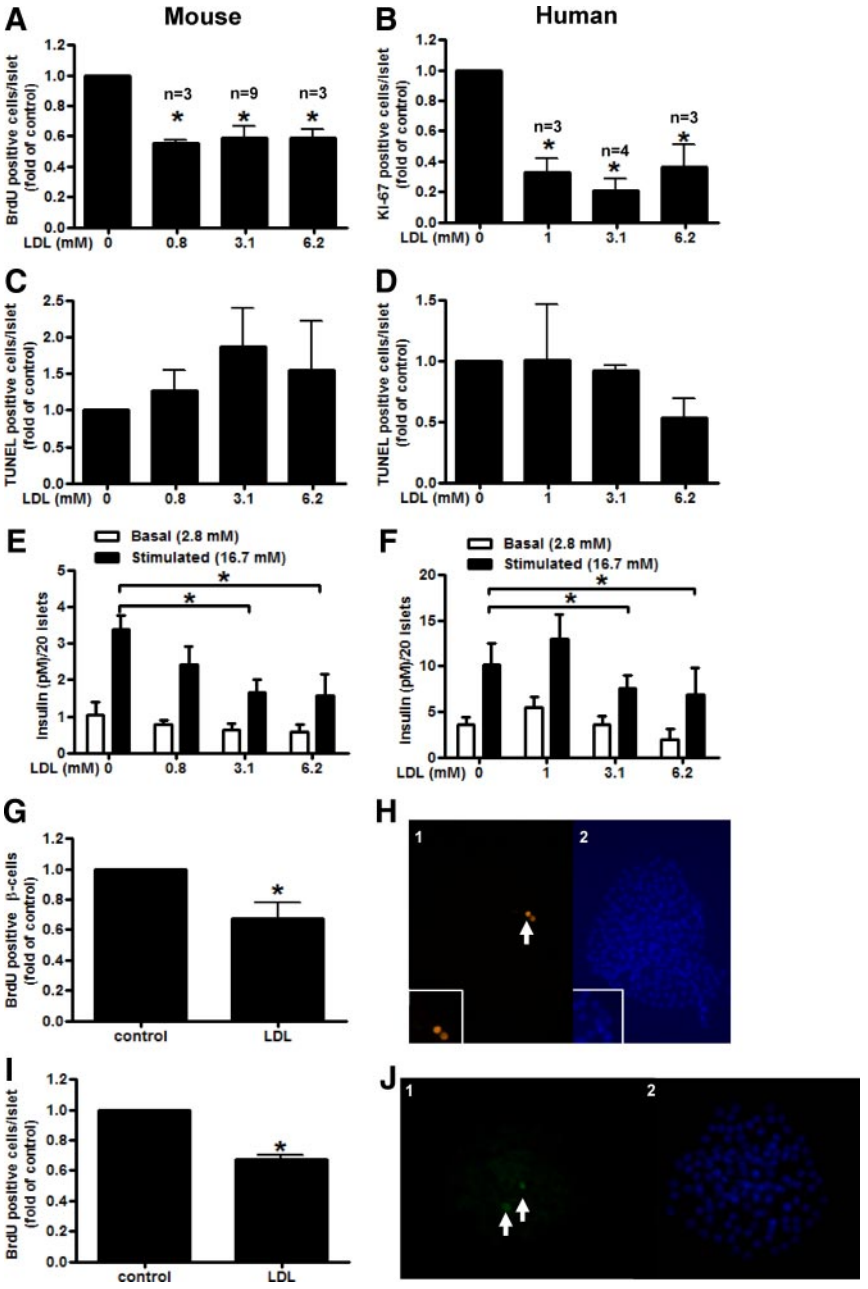

FIG. 1. LDL decreases basal proliferation and function of mouse and human islet cells. Mouse islets (A, C, E, G, and I) or human islets (B, D, and F) were cultured on extracellular matrix-coated dishes for 2 (I) or 4 (A–G) d at 11 mM (mouse) or 5 mM (human) glucose in the presence of increasing concentrations of LDL. A, BrdU-positive mouse islet cells per islet (control, 3 ± 0.3, n = 3–9). B, Ki-67-positive human islet cells per islet (control, 0.15 ± 0.05, n = 3–4). C, TUNEL-positive mouse islet cells per islet (control, 0.7 ± 0.3, n = 6). D, TUNEL-positive human islet cells per islet (control, 0.34 ± 0.1, n = 3–4). E, Glucose-stimulated insulin secretion in mouse islets (n = 5). F, Glucose-stimulated insulin secretion in human islets (n = 5). G, Mouse islets were dispersed into single cells and cultured as single cells for 4 d on extracellular matrix-coated dishes in the absence (control) or presence of LDL (3.1 mM). BrdU-positive mouse β-cells (control, 0.14 ± 0.07, n = 6). H, Picture of a representative mouse islet costained for BrdU (1; *arrows*) and DAPI (2). I, BrdU-positive mouse islet cells per islet (control, 3.8 ± 1.3, n = 3). J, Picture of a representative mouse islet costained for TUNEL (1; *arrows*) and DAPI (2). *, $P < 0.05$ as tested by ANOVA followed by Bonferroni *post hoc* test (A-D) or by Student's *t* test (E–G).

ment of maximal glucose-stimulated insulin secretion by LDL was observed in wild-type islets but not islets of $LDLR^{-/-}$ mice (Fig. 2, C–E). In both strains, the presence of LDL did not affect insulin content ($LDLR^{+/+}$: control, 188 ± 43 μg/liter per 20 islets; LDL, 181 ± 81 μg/liter per 20 islets, n = 3, n.s.; $LDLR^{-/-}$: control, 279 ± 93 μg/liter per 20 islets; LDL, 134 ± 38 μg/liter per 20 islets, n = 3, n.s.) or insulin mRNA expression ($LDLR^{+/+}$: control, reference = 1; LDL, 0.9 ± 0.4, n = 2; $LDLR^{-/-}$: control, reference = 1, LDL, 1.2 ± 0.5, n = 2).

HDL decreases basal apoptosis of islet cells

Human and mouse islets were cultured on extracellular matrix-coated plates for 4 d in the presence of various concentrations of HDL. Exposure to 0.8 mM HDL-C (as defined by cholesterol concentration) induced a 30% decrease in mouse islet cell apoptosis (Fig. 3C) without affecting proliferation (Fig. 3A) or glucose-stimulated insulin secretion (Fig. 3E). Lower HDL concentrations did not affect function, proliferation, or apoptosis of mouse islets. Similarly, exposure of human islets to 0.5 or 1 mM HDL-C induced a 30–60% decrease in islet cell apoptosis (Fig. 3D) and did not affect proliferation or glucose-stimulated insulin secretion (Fig. 3, B and F). Based on the dose-response results, all subsequent experiments on mouse and human islets were performed with HDL at concentrations of 0.8 and 1 mM HDL-C, respectively.

HDL protects β-cells against glucose or IL-1β-induced apoptosis

To further assess the antiapoptotic effect of HDL, human and mouse islets were cultured on extracellular matrix-coated plates for 4 d in the presence of elevated glucose concentrations and 0.8 mM (mouse) or 1 mM (human) HDL-C. As previously reported (20), glucose induced a 2-fold increase in mouse and human islet cells apoptosis (Fig. 4, A and B). Interestingly, HDL was able to prevent the apoptosis induced by the presence of elevated glucose concentrations in both human and mouse islets. Moreover, HDL protected mouse islet cells from IL-1β-induced apoptosis after

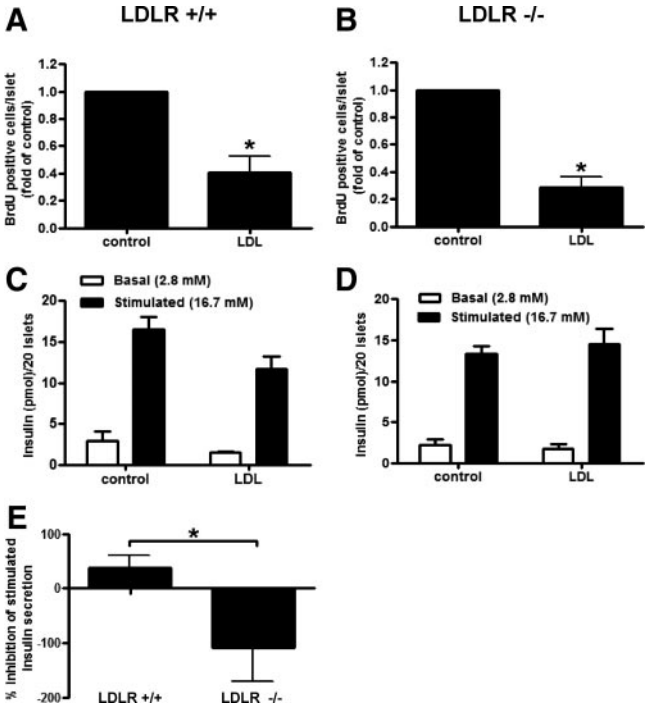

FIG. 2. LDL decreased islet cell proliferation is independent of the LDLR. Mouse islets from $LDLR^{+/+}$ (A and C) or $LDLR^{-/-}$ (B and D) mice were cultured for 4 d in 11 mM glucose on extracellular matrix-coated dishes in the absence (control) or presence of LDL (3.1 mM). A, BrdU-positive cells per wild-type islet in the absence (control) or presence of LDL (3.1 mM) (control, 1.8 ± 1, n = 3). B, BrdU-positive cells per $LDLR^{-/-}$ islet in the absence (control) or presence of LDL (3.1 mM) (control, 1.4 ± 0.5, n = 3). C, Glucose-stimulated insulin secretion in wild-type mouse islets in the absence (control) or presence of LDL (3.1 mM) (n = 3). D, Glucose-stimulated insulin secretion in $LDLR^{-/-}$ mouse islets in the absence (control) or presence of LDL (3.1 mM) (n = 3). E, Percent inhibition of stimulated insulin secretion (n = 3). *, $P < 0.05$ as tested by Student's *t* test.

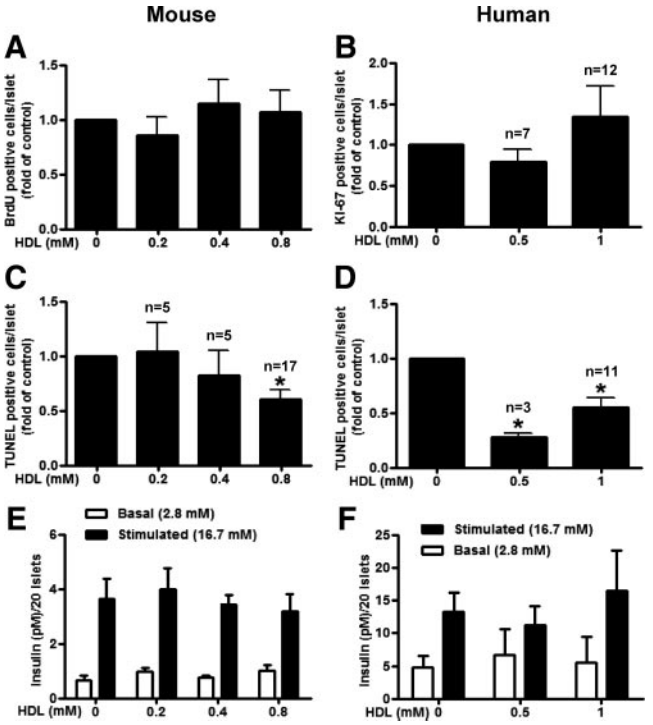

FIG. 3. HDL decreases basal apoptosis of mouse and human islet cells. Mouse and human islets were cultured on extracellular matrix-coated dishes for 4 d at 11 mM (mouse) or 5 mM (human) glucose in the presence of increasing concentrations HDL. A, BrdU-positive mouse islet cells per islet (control, 5.1 ± 1.2, n = 5). B, Ki-67-positive human islet cells per islet (control, 0.07 ± 0.03, n = 7–12). C, TUNEL-positive mouse islet cells per islet (control, 1.7 ± 0.7, n = 5–17). D, TUNEL-positive human islet cells per islet (control, 0.33 ± 0.04, n = 4–11). E, Glucose-stimulated insulin secretion in mouse islets (n = 5). F, Glucose-stimulated insulin secretion in human islets (n = 5). *, $P < 0.05$ as tested by ANOVA followed by Bonferroni *post hoc* test.

2 d in culture (Fig. 4C). To determine which islet cell type is protected by HDL against IL-1β-induced apoptosis, mouse islets were exposed for 2 d to IL-1β alone or with IL-1β and HDL to be then analyzed for apoptosis with confocal microscopy. By colocalization, this method allowed us to determine whether the apoptotic cells were insulin positive (β-cells) or not (non-β-cells). Indeed, HDL protected mouse β-cells from IL-1β-induced apoptosis, with no effect on non-β-cells (Fig. 4, D–F).

β-Cell protection against IL-1β-induced apoptosis is independent of SRB1

In an attempt to uncover which receptor is involved in the protection of β-cells by HDL against IL-1β-induced apoptosis, islets obtained from $SRB1^{-/-}$ or wild-type mice were exposed for 2 d to IL-1β alone or in the presence of HDL. SRB1 is known to be expressed on mouse islets (6). The islets were analyzed for apoptosis by confocal microscopy, allowing the identification of apoptotic β-cells. HDL protected β-cells from IL-1β-induced apoptosis in wild-type and $SRB1^{-/-}$ islets to the same degree, although the antiapoptotic effect was not statistically significant in the latter (Fig. 5, A and B).

Both ApoA1 and S1P protect islet cells from IL-1β and glucose-induced apoptosis

To determine which HDL component is mediating the protective effect against IL-1β and glucose-induced apoptosis, HDL was delipidated. Mouse islets were treated with either the lipid-free protein moiety (pHDL) or the protein-free lipid moiety of HDL. Similarly to HDL, the pHDL protected mouse islet cells from glucose-induced apoptosis (Fig. 5C). Likewise, 20 μg/ml purified ApoA1, which constitutes the major protein in HDL protected mouse islets from glucose-induced apoptosis (Fig. 5D). In addition, 20 μg/ml lipid-free ApoA1 also inhibited the apoptosis of mouse islets induced by IL-1β (Fig. 5E). Surprisingly, the lipid moiety of HDL also protected islets from IL-1β-induced apoptosis after 2 d (Fig. 5F). One micromole S1P, a bioactive sphingolipid carried by HDL, also protected mouse islet cells from IL-1β-induced apoptosis (Fig. 5G).

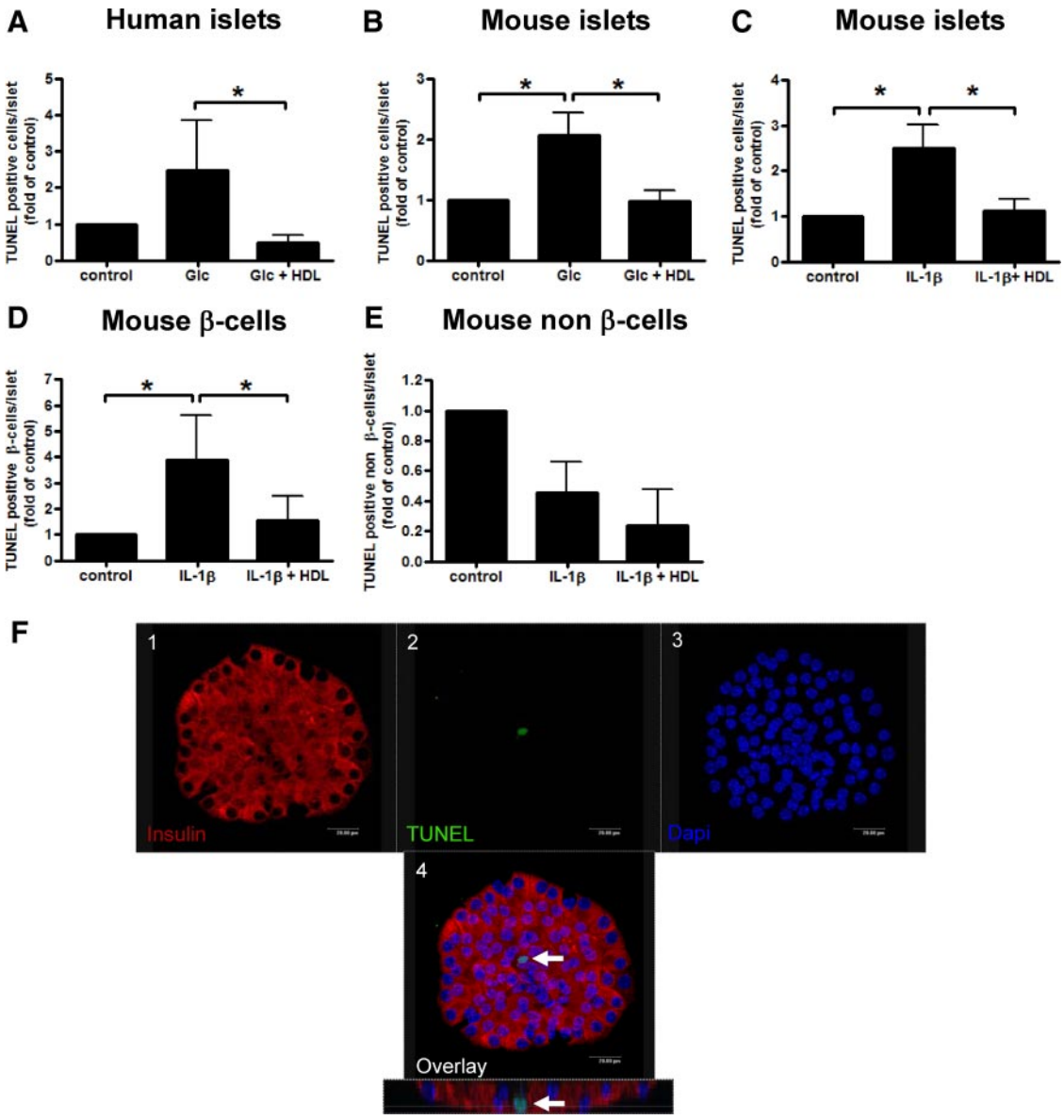

FIG. 4. HDL protects mouse β-cells from IL-1β-induced apoptosis. Islets were cultured for 2 (C, D, and E) or 4 d (A and B) on extracellular matrix-coated dishes. A, TUNEL-positive human islet cells per islet after 4 d culture in 5 (control) or 33 mM glucose (Glc) in the absence (control) or presence of HDL (1 mM) (control, 0.1 ± 0.05, n = 4). B, TUNEL-positive mouse islet cells per islet after 4 d culture in 11 (control) or 33 mM glucose (Glc) in the absence (control) or presence of HDL (0.8 mM) (control, 0.45 ± 0.1, n = 7). C, TUNEL-positive mouse islet cells per islet after 2 d culture in 11 mM glucose in the absence (control) or presence of IL-1β (2 ng/ml) and the absence (control) or presence of HDL (0.8 mM) (control, 0.46 ± 0.1, n = 16). D, TUNEL-positive β-cells per islet after 2 d culture in 11 mM glucose in the absence (control) or presence of IL-1β (2 ng/ml) and the absence (control) or presence of HDL (0.8 mM) (control, 0.34 ± 0.2, n = 3). E, TUNEL-positive non-β-cells per islet after 2 d culture in 11 mM glucose in the absence (control) or presence of IL-1β (2 ng/ml) and the absence (control) or presence of HDL (0.8 mM) (control, 0.47 ± 0.2, n = 3). F, Confocal microscope pictures of a representative islet costained for insulin (1), TUNEL (2), and Dapi (3). The *arrows* point to a cell positive for all three of these. Pictures of the different stainings on planes XZ and the overlay (4, XZ and XY planes) are shown. *, $P < 0.05$ as tested by ANOVA followed by Bonferroni *post hoc* test.

Therefore, both the protein and lipid moieties of HDL displayed antiapoptotic effects on islets.

HDL modulates iNOS, Fas, and FLIP mRNA expression

Finally, we investigated the mechanisms underlying the antiapoptotic effect of HDL in mouse islets. Incubation of mouse islets with 0.8 mM HDL-C for 2 d strongly decreased basal mRNA expression of iNOS (Fig. 6A) as well as the extent of IL-1β-induced iNOS mRNA expression (Fig. 6B). In addition, after 4 d incubation, HDL was found to modulate the expression of Fas and FLIP, two molecules involved in the extrinsic apoptosis pathway. Indeed, HDL down-regulated the expression of the death receptor Fas by 30% and up-regulated FLIP, an inhibitory protein of the Fas pathway, by 50% (Fig. 6, C and D). By contrast HDL did not modulate the expression of SOCS3 and Bcl2, two other

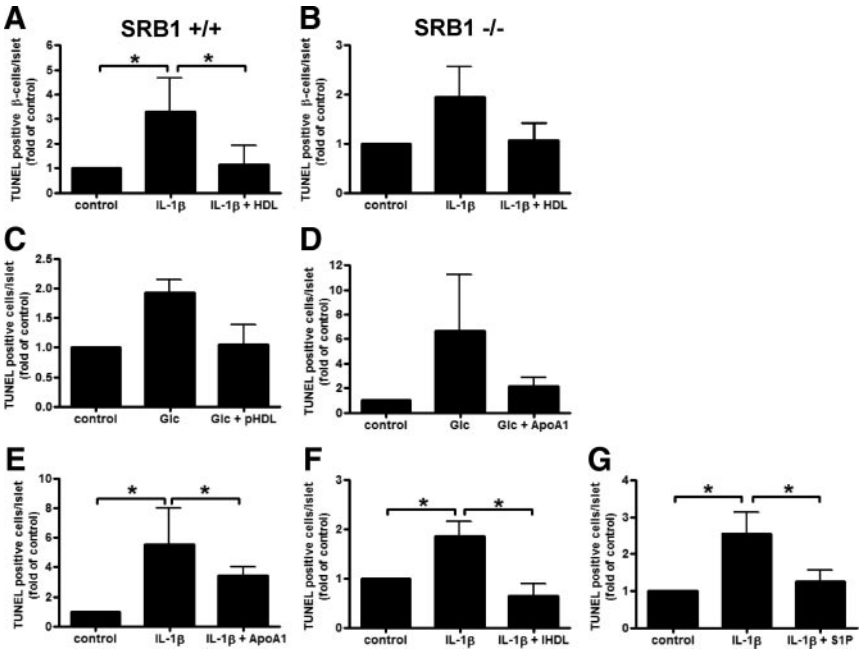

FIG. 5. HDL-mediated β-cell protection against IL-1β-induced apoptosis is independent of SRB1 expression. ApoA1 and S1P mediate the protective effect of HDL on islet cell apoptosis. Islets were cultured for 2 (A, B, E, F, and G) or 4 d (C and D) on extracellular matrix-coated dishes. A, TUNEL-positive β-cells per wild-type islet after 2 d culture in 11 mM glucose in the absence (control) or presence of IL-1β (2 ng/ml) and the absence (control) or presence of HDL (0.8 mM) (control, 0.34 ± 0.13, n = 4). B, TUNEL-positive β-cells per SRB1$^{-/-}$ islet after 2 d culture in 11 mM glucose in the absence (control) or presence of IL-1β (2 ng/ml) and the absence (control) or presence of HDL (0.8 mM) (control, 0.5 ± 0.2, n = 3). C, TUNEL-positive mouse islet cells per islet after 4 d culture in 11 (control) or 33 mM glucose (Glc) in the absence (control) or presence of pHDL (20 μg/ml) (control, 0.31 ± 0.1, n = 4). D, TUNEL-positive mouse islet cells per islet after 4 d culture in 11 (control) or 33 mM glucose (Glc) in the absence (control) or presence of ApoA1 (20 μg/ml) (control, 0.17 ± 0.1, n = 3). E, TUNEL-positive mouse islet cells per islet after 2 d culture in 11 mM glucose in the absence (control) or presence of IL-1β (2 ng/ml) and the absence (control) or presence of ApoA1 (20 μg/ml) (control, 0.12 ± 0.06, n = 3). F, TUNEL-positive mouse islet cells per islet after 2 d culture in 11 mM glucose in the absence (control) or presence of IL-1β (2 ng/ml) and the absence (control) or presence of HDL lipid moiety (lHDL; 0.8 mM) (control, 0.6 ± 0.2, n = 3). G, TUNEL-positive mouse islet cells per islet after 2 d culture in 11 mM glucose in the absence (control) or presence of IL-1β (2 ng/ml) and the absence (control) or presence of S1P (1 μM) (control, 1 ± 0.3, n = 3). *, $P < 0.05$ as tested by ANOVA followed by Bonferroni *post hoc* test.

molecules involved in the apoptotic process (Fig. 6, E and F).

Discussion

In this study of primary mouse and human islets, we found that LDL exerts potentially diabetogenic effects by decreasing both maximal insulin secretion and proliferation of β-cells, whereas HDL exerts potentially antidiabetogenic effects by inhibiting β-cell apoptosis.

Potentially diabetogenic effects of LDL on islets

LDL tended to decrease maximal glucose-stimulated insulin secretion from murine and human islets at clinically relevant concentrations. We hypothesize that LDL affects only the secretory machinery and not the production of insulin because no difference was observed in either total insulin content or insulin mRNA level between LDL-treated and untreated islets. The LDLR appears to play a central role in mediating these adverse effects because LDL did not impair maximal insulin secretion from islets of LDLR$^{-/-}$ mice. In agreement with a limiting effect of the LDLR on β-cell function, it has been reported that patients with heterozygous LDLR deficiency are at reduced risk for developing diabetes mellitus (21, 22). Conversely, the increased risk of diabetes mellitus observed in patients treated with lipophilic statins, which can enter β-cells, may be explained by statin-induced up-regulation of the LDLR (23, 24). Because the LDLR acts as an endocytic receptor, we hypothesize that the impairment of maximal glucose-stimulated insulin secretion by LDL is mediated by a component of LDL, which is internalized by the LDLR pathway. Possibly this component is cholesterol because impaired cholesterol homeostasis in islets, *i.e.* of mice with a β-cell-specific knockout of the ATP-binding cassette transporter A1 and hence disturbed cholesterol efflux, was previously shown to contribute to islet dysfunction (9). Similar to our *ex vivo* model, cholesterol accumulation in β-cells led to impaired insulin secretion without affecting insulin production (7, 25). Conversely, lowering cholesterol levels in β-cells with methyl-β-cyclodextrin, which depletes membrane cholesterol, or with a statin, which inhibits *de novo* synthesis, was found to improve the secretory function of β-cells and increase insulin secretion (25). However, the mechanism by which cholesterol accumulation in β-cells interferes with glucose-stimulated insulin secretion remains to be further investigated.

In parallel with impairing the maximal insulin secretion of β-cells, LDL also decreased the basal proliferation of mouse and human islet cells. This finding is in agreement with previous findings in βTC3 cells in which elevated LDL concentrations decreased cell proliferation (6, 26). Moreover, in contrast to the inhibitory effect of LDL on insulin secretion, the antiproliferative effect of LDL was

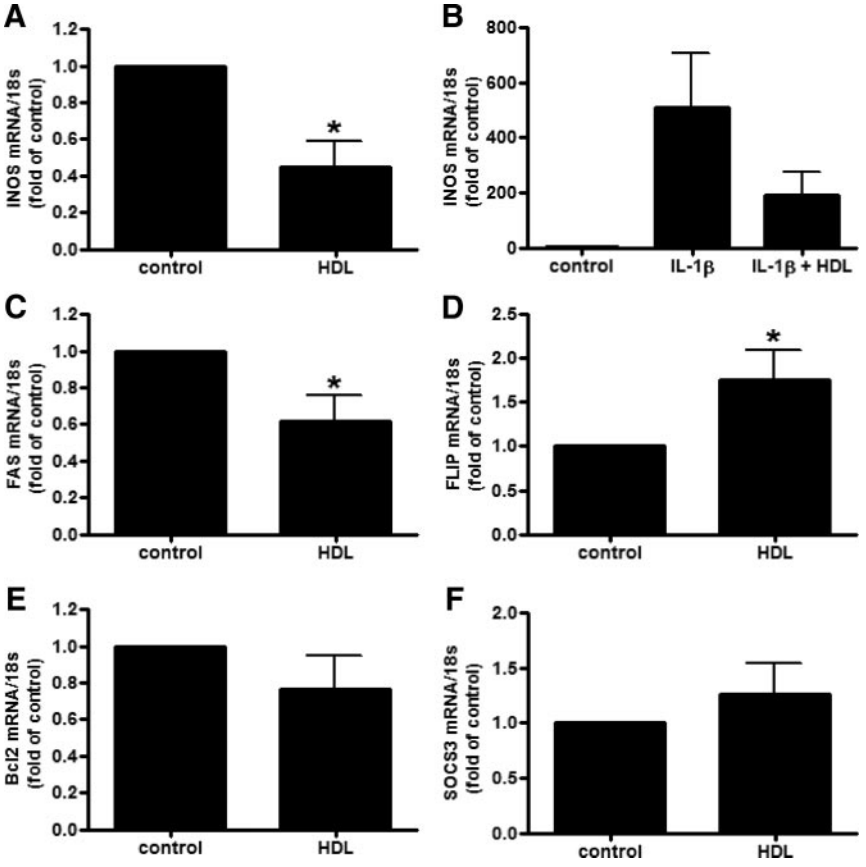

FIG. 6. HDL down-regulates Fas and iNOS and up-regulates FLIP mRNA expression. Mouse islets were cultured for 2 or 4 d on extracellular matrix-coated dishes before RNA extraction. Semiquantitative analysis of gene expression was performed with the TaqMan technology. A and B, iNOS mRNA expression after 2 d incubation in the absence (control) or presence of HDL (0.8 mM) and with or without IL-1β (2 ng/ml) (A and B: n = 5). C–F, Fas (C), FLIP (D), Bcl2 (E), and SOCS3 (F) mRNA expression after 4 d incubation in the absence (control) or presence of HDL (0.8 mM) (C: n = 7; D: n = 6; E: n = 8; F: n = 5). *, $P < 0.05$ as tested by Student's *t* test.

not abolished in islets of LDLR$^{-/-}$ mice and hence appears to be independent of LDL uptake by the LDLR. This does not exclude that internalization of cholesterol or other LDL components interferes with β-cell proliferation because β-cells express several receptors that mediate the internalization of cholesterol with LDL, *e.g.* ApoER2 (6). In addition, scavenger receptors for modified LDL must be considered as candidates, although we took several measures to prevent lipoprotein modification during the experiments: antioxidants were added to the culture media and the LDL containing cell culture media were discarded and replaced with fresh lipoproteins every day during the culture period. Because the antiproliferative effect of LDL was still observed under these conditions, we assume that native rather than oxidatively modified LDL is responsible for this antiproliferative effect.

In contrast to Roehrich *et al.* (6) but in agreement with Abderrahmani *et al.* (8), we did not observe any significant effect of native LDL on the survival of either mouse or human islet cells. However, Roehrich *et al.* (6) used higher concentrations of LDL than we did. Moreover, Abderrahmani *et al.* (6, 8) found that oxidatively modified LDL induces apoptosis of β-cells. It may hence well be that in addition to the adverse effects on function and proliferation LDL has negative impact also on β-cell survival when it is modified or present at very high concentrations.

Potentially antidiabetogenic effects of HDL on islets

In the present study and in agreement with the data from Roehrich *et al.* (6), we found that HDL decreases basal human and mouse islet cell apoptosis without influencing the function or the proliferation of these cells. Moreover, HDL protected islet cells from glucose and IL-1β-induced apoptosis. These protective effects were specific for β-cells as indicated by our immunological colocalization experiments. The protective effects exerted by HDL suggest a modulating role for this particle on the survival of β-cells and hence in the pathogenesis of type 2 diabetes.

Because HDL is a very heterogeneous class of lipoproteins that contains more than 50 bioactive proteins (27) and even more lipid species (28), we attempted to uncover the component of HDL affecting islet cell survival. Surprisingly, both the protein and lipid moieties of HDL were found to protect islet cells against glucose-induced apoptosis. Notably, purified ApoA1, the main protein constituent of HDL, and S1P protected islet cells from IL-1β and glucose-induced apoptosis. These results are in agreement with a previously published study in which S1P protected a rat β-cell line against cytokines-induced apoptosis (29). Interestingly, both ApoA1 and S1P were previously also found to exert antiinflammatory and antiapoptotic effects on endothelial cells (30). In endothelial cells the antiapoptotic effect of S1P involves the phosphorylation of endothelial nitric oxide synthase, which, however, is not expressed in β-cells. Rather, the iNOS is expressed, which is known to mediate the proapoptotic effects of glucose and cytokines (31). Interestingly exposure of mouse islets to HDL down-regulated iNOS mRNA expression as well as downstream target of activated iNOS, namely Fas, whereas the antiapoptotic FLIP was up-regulated.

Both S1P receptors and the HDL receptor SRB1 have been shown to mediate the antiapoptotic effects of HDL on endothelial cells (32, 33) and to be expressed on β-cells (6, 34). The role of SRB1 in the mediation of the protection of islet cells against IL-1β-induced apoptosis by HDL is not clear. Our data suggest that SRB1 is probably not involved in the inhibition of IL-1β-induced apoptosis by HDL because the inhibitory effect of HDL was similar in islets from $SRB1^{-/-}$ and wild-type mice, although only the effect in wild-type islets was statistically significant.

In conclusion and to the best of our knowledge, we showed for the first time that LDL and HDL influence the survival and function of not only transformed β-cell lines or primary murine β-cells but also primary human islets. In addition, we showed that the effects of LDL on insulin secretion and β-cell proliferation are exerted by different pathways, namely dependently and independently, respectively, of the LDLR. Finally, we identified two components of HDL, ApoA1 and S1P, by which HDL protect β-cells from cytokine- or glucose-induced apoptosis. Together with data from other laboratories and epidemiological studies, these findings point to the relevance of both lipoprotein classes for the pathogenesis of diabetes mellitus. The data provide a rationale for future studies to show that a good control of LDL and HDL plasma concentrations prevents not only atherosclerosis as an important complication but also the onset and progression of type 2 diabetes.

Acknowledgments

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